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Abstract: Xylitol has been claimed to reduce mutans streptococci (MS) in dental plaque by energy-consuming futile metabolic cycles. This study aimed to investigate the effects of xylitol on MS in an in vitro 6-species oral biofilm model. Each multispecies biofilm contained either a laboratory reference strain, a fresh isolate, a xylitol-sensitive or a xylitol-resistant strain of Streptococcus mutans or Streptococcus sobrinus . Biofilms, grown on pellicle-coated hydroxyapatite discs, were fed with a glucose/sucrose-supplemented medium 3 times daily for 45 min and incubated in saliva between feedings. Before or after feeding, biofilms were exposed to either 7.5% xylitol, 7.5% sorbitol or to saliva (control) for 20 min. After 64.5 h, biofilms were harvested and the microbial composition was analysed by non-selective and selective culturing. Strain variability in the ability to colonize biofilms was observed. However, the response patterns in the biofilms to the 4 polyol treatments were similar. None of the MS were inhibited by xylitol provided either before or after feeding. Sorbitol given before feeding did not affect microbial growth whereas sorbitol provided after feeding showed a slight, albeit statistically significant increase in MS counts for some of the tested strains. It did so at the expense of Streptococcus oralis, which decreased in numbers. The present findings do not support the contention that xylitol reduces MS in plaque by futile metabolic cycles.

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Effects of xylitol on survival of mutans streptococci in mixed-six-species in vitro biofilms modelling supragingival plaque

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Declaration of Interests

None of the authors have any personal or financial interests related to the content of this paper. The study was carried out of pure scientific interest to elucidate further the effect of xylitol on mutans streptococci. It was financed by the Universities of Bergen and Zürich and by the Swedish Patent Revenue Fund for Research in Preventive Odontology.

Abstract

Xylitol has been claimed to reduce mutans streptococci (MS) in dental plaque by energy-consuming futile metabolic cycles. This study aimed to investigate effects of xylitol on MS in an in vitro six-species oral biofilm model. Each multispecies biofilm contained either a laboratory reference strain, a fresh isolate, a xylitol-sensitive or a xylitol-resistant strain of *Streptococcus mutans* or *Streptococcus sobrinus*. Biofilms, grown on pellicle-coated hydroxyapatite discs, were fed with a glucose/sucrose-supplemented medium three times daily for 45 min and incubated in saliva between feedings. Before or after feeding, biofilms were exposed to either 7.5% xylitol, 7.5% sorbitol or to saliva (control) for 20 min. After 64.5 h biofilms were harvested and the microbial composition was analyzed by non-selective and selective culturing. Strain variability in the ability to colonize biofilms was observed. However, the response patterns in the biofilms to the four polyol treatments were similar. None of the MS were inhibited by xylitol provided either before or after feeding. Sorbitol given before feeding did not affect microbial growth whereas sorbitol provided after feeding showed a slight, albeit statistically significant increase in MS counts for some of the tested strains. It did so at the expense of *Streptococcus oralis,* which decreased in numbers. The present findings do not support the contention that xylitol reduces MS in plaque by futile metabolic cycles.

Introduction

The naturally occurring sugar alcohols xylitol and sorbitol, and combinations thereof, have been widely used as sugar substitutes in chewing gums and confectionary products [Bär, 1988; Birkhed and Bär, 1991; Bowen, 1996; Van Loveren, 2004]. Xylitol is non-cariogenic since it is not fermented by most oral microorganisms and does not give rise to acid formation in dental plaque [Edwardsson et al., 1977; Birkhed and Edwardsson, 1978; Havenaar et al., 1978; Hayes and Roberts, 1978; Bär, 1988; Van Loveren, 2004]. Sorbitol is slowly fermented by comparatively few microorganisms in plaque [Edwardsson et al., 1977; Havenaar et al., 1978; Kalfas and Edwardsson, 1990], and products formulated with sorbitol are accepted as being non-cariogenic [Birkhed and Bär, 1991; Bowen, 1996; Van Loveren, 2004].

Besides its non-cariogenicity, xylitol has attracted much attention as a caries-preventive or even caries-therapeutic agent. The mechanisms behind these disputed properties have yet to be defined. It has been claimed that xylitol reduces the acidogenic potential of plaque, suppresses growth of plaque bacteria, notably of mutans streptococci (MS), enhances salivation and promotes remineralization of dental hard tissues [Tanzer, 1995; Twetman and Holgerson, 2004].

The inhibtory effects of xylitol on oral microorganisms have been attributed mainly to its interference with MS [Bär, 1988; Tanzer, 1995; Trahan, 1995]. The mechanism of action was suggested to be an energy-consuming cycle related to the uptake and phosphorylation of xylitol to xylitol 5-phosphate and the subsequent dephosphorylation and expulsion of xylitol [Assev and Rølla, 1984; Trahan et al., 1985; Assev and Rølla, 1986; Trahan et al., 1991]. Intracellular accumulation of xylitol 5-phosphate conceivably leads to inhibition of bacterial glycolytic enzymes, resulting in impaired growth and acid production, as well as an ecological selection towards xylitol-resistant (X-R) MS in plaque [Assev and Rølla, 1984; Trahan et al., 1985; Assev and Rølla, 1986; Trahan and Mouton, 1987; Trahan et al., 1991, 1992; Trahan,

1995]. The X-R strains seem to become inert to xylitol, so that their glycolysis is no longer inhibited by xylitol [Gauthier et al., 1984].

It is well documented that the continuous presence of xylitol in the culture medium of planktonically grown monocultures inhibits the in vitro growth of MS and some other oral bacterial species [Assev et al., 1983; Assev and Rølla, 1984; Trahan et al., 1985; Assev and Rølla, 1986; Trahan et al., 1991]. An intriguing question is, however, whether the so-called "futile xylitol cycle" – uptake and expulsion of xylitol under expenditure of energy – operates in dental plaque. This has not been substantiated. A previously developed mixed-six-species biofilm model of supragingival plaque based on a batch culture approach [van der Ploeg and Guggenheim, 2004; Thurnheer et al., 2006] seemed to be a valuable tool to investigate the effects of xylitol on survival of MS in biofilms containing various strains of xylitol-sensitive (X-S), X-R, fresh isolates or laboratory types of MS. For comparison, sorbitol was included as an additional test substance since it is only slowly metabolized by some oral microorganisms without any claims of "specific effects" as a cariostatic agent [Birkhed and Bär, 1991; Bowen, 1996; Van Loveren, 2004]. The aim of the present study was to test the hypothesis that repeated exposures of mixed-six-species oral biofilms in vitro to xylitol reduce the numbers of MS through futile metabolic cycles.

Material and Methods

Inoculum and media preparation

A modification of the Zürich in vitro model of supragingival plaque [Guggenheim et al., 2001] was adopted in this experiment. For biofilm formation, the following species were used: *Actinomyces naeslundii* (OMZ 745), *Candida albicans* (OMZ 110), *Fusobacterium nucleatum* (OMZ 596), *Streptococcus oralis* (SK 248), *Veillonella dispar* (ATCC 17748) and one of the MS listed in table 1.

All strains were cultivated on Columbia Blood Agar (Difco, Sparks, MD, USA). Colonies of each strain were transferred to 9 ml of modified fluid universal medium (mFUM) containing 0.3% of glucose (plus 1% sodium lactate, for *V. dispar*) and anaerobically incubated at 37 ºC. mFUM corresponds to a well established tryptone-yeast based broth medium designated as FUM [Gmür and Guggenheim, 1983] and modified by supplementing 67 mmol/l Sørensen's buffer (final pH 7.2). After approximately 15 h of incubation, 200 µl of each suspension were transferred to 5 ml of a fresh mFUM (containing 0.3% glucose) and further incubated until an optical density of 1.0 ± 0.02 at 550 nm was reached. Aliquots from *A. naeslundii*, *C. albicans*, *F. nucleatum*, *S. oralis*, *V. dispar* and one of the *S. mutans* or *S. sobrinus* strains (table 1), were combined, in order to form a standardized mixed-six-species suspension, and stored on ice until the onset of the biofilm experiment [Guggenheim et al., 2001]. In all steps mentioned above, the *C. albicans* strain was incubated in 10% CO_2.

The biofilms were formed on sterile hydroxyapatite discs (Ø 10.6 mm; Clarkson Chemical Company, Williamsport, PA, USA). For each mixed-six-species biofilm experiment, 15 hydroxyapatite discs were used. In order to form a salivary pellicle on the surface of the discs, they were placed in 24-well polystyrene cell culture plates and covered with 1600 µl of processed saliva prepared as described previously [Guggenheim et al., 2001]. During pellicle formation the plates were gently shaken at room temperature for 4 h.

Time flow of "feast and famine" biofilm experiments

An overall survey of the time flow of each biofilm experiment is provided in fig. 1. Throughout this study, a "feast and famine" protocol [van der Ploeg and Guggenheim, 2004; Thurnheer et al., 2006] was adopted. After salivary pellicle formation, saliva was replaced at time *0 h* with a mixture of processed saliva (480 µl) and mFUM (1120 µl) containing 0.3% glucose (fig. 1). In each well, a volume of 200 µl of mixed-six-species suspension was added

and the culture plate was anaerobically incubated at 37 ºC for 45 min. Then, the discs were dip-washed three times in physiological saline, transferred to new wells containing 1600 µl fresh processed saliva and incubated anaerobically at 37 ºC up to time point *16.5 h* (period of famine). At time points *16.5 h, 20.5 h, 24.5 h, 40.5 h, 44.5 h*, and *48.5 h* all discs were re-exposed for 45 min to a cell-free mixture of processed saliva (480 µl) and mFUM (1120 µl) with 0.15% glucose + 0.15% sucrose (periods of feast), after which they were dip-washed as described above and transferred back into processed saliva (famine). After the feeding periods starting at *16.5 h, 20.5 h, 40.5 h, and 44.5 h* the discs were put back into the same processed saliva provided before feeding, whereas after the feeding periods starting at *24.5 h* and *48.5 h* the discs were transferred to new plates with fresh processed saliva. Experiments were stopped and biofilms analysed after 64.5 h of incubation. Exposures to polyols lasted for 20 min and occurred immediately before or immediately after feeding periods as described in the next section.

Exposure of biofilms to polyol solutions

Figure 2 provides a schematic overview of the allocation of biofilms to the different treatments within one experiment. Every experiment covered one of the eight mutans strains and included triplicate biofilms for each treatment. In order to evaluate the effect of the polyols on starved biofilms three discs were exposed anaerobically (37 °C, 20 min) before each feeding period to a mixture of processed saliva (480 µl) and water (1120 µl) containing either 7.5% xylitol (discs 4-6) or 7.5% sorbitol (discs 7-9). These polyol percentages indicate final concentrations in the entire 1600 µl. During this time the remaining nine discs (discs 1-3, 10-15) were put into a corresponding mixture of fresh processed saliva and water. Thereafter, the biofilms were 3x dip-washed in saline and then transferred to new wells for the feeding period.

To evaluate the effect of polyols on MS after feeding, another three discs were transferred to a fresh mixture of processed saliva and water with either 7.5% xylitol (discs 10-12) or 7.5% sorbitol (discs 13-15) (fig. 2), while all remaining discs were switched to processed saliva/water as described above. After 20 min of anaerobic incubation at 37 ºC, all discs were dip-washed again three times in physiological saline, transferred to new wells containing processed fresh saliva and incubated anaerobically at 37 ºC (next famine period). During the entire experiment three control discs (discs 1-3) were never exposed to polyol solutions (fig. 2).

In total, 120 biofilms were used in this study: eight types of biofilm each with a different strain of MS, five treatments per biofilm type (control, xylitol and sorbitol before and after feeding), and three replicate discs for every single treatment.

Harvesting and determination of colony forming units

After 64.5 h, the biofilms were dip-washed in saline as described above to remove loosely adherent bacteria. Then the surface of each disc was scraped with a plastic scaler (KerrHawe SA, CH-6934 Bioggo, Switzerland) to remove the entire biofilm and the cells were collected in 1 ml saline. The cell suspension was then sonicated (5 s, 30 W) and aliquots were plated out using a spiral diluter. Total CFUs were assessed on Columbia Blood Agar plates. Total streptococci and all streptococcal species were assessed on Mitis Salivarius Agar (Difco). Columbia Blood Agar plates were incubated anaerobically and Mitis Salivarius Agar plates in 10% CO_2 at 37 ºC. After 72 h of incubation, all CFUs were counted under a stereomicroscope and the results were expressed as total CFUs, total streptococci CFUs, *S. oralis* CFUs and *S. mutans* or *S. sobrinus* CFUs. In order to allow a comparison of the polyol treatments between biofilms containing different MS strains, the results were expressed as

delta CFUs, calculated by the difference in CFUs between each polyol treatment and its respective control group.

Statistics

Descriptive statistics of the data were performed with StatView 5.01 (SAS Institute, Cary, NC, USA) and illustrated with box plots. The $\log_{10}$-transformed data met the requirements for parametric analysis. Hence differences between treatments were analyzed by ANOVA followed by the Scheffe test (significance level $p < 0.05$).

Results

In total, eight mixed-six-species biofilms were grown in the present study, each one containing only one of the eight MS strains listed in table 1. Xylitol did not inhibit growth of any of the X-S or X-R MS strains tested in mixed-six-species biofilms whether it was provided before or after feeding (tables 2-5, fig. 3).

In the presence of the X-S MS strain *S. sobrinus* ATCC 27352 in mixed-species biofilms, higher *S. sobrinus* counts were found in biofilms treated with sorbitol after feeding than in biofilms from the control group (table 2). The biofilms containing the *S. mutans* CM7 X-S strain and treated with sorbitol after feeding, showed higher total CFUs and higher total streptococci CFUs when compared to controls or to biofilms treated with xylitol before feeding. These biofilms also showed higher *S. mutans* CFUs than the controls and the biofilms exposed to xylitol before or after feeding. In addition, exposure to sorbitol after feeding resulted in lower counts of *S. oralis* compared to the administration of sorbitol before feeding (table 2).

In biofilms harbouring the X-R mutans strains no effect of polyols was observed, except in presence of the CM7 X-R strain where the biofilms treated with sorbitol after

feeding showed higher counts of *S. mutans* and lower counts of *S. oralis* compared to the other groups (table 3).

With biofilms containing the fresh isolates of *S. mutans* (P-03 and M-51), no polyol effect was observed, except for the one on M-51 positive biofilms, which showed significantly reduced counts of *S. oralis* when treated with sorbitol after feeding (table 4). No polyol effects on total CFUs, total streptococcal CFUs and *S. oralis* CFUs were observed in the presence of the laboratory reference strains OMZ 176 (*S. sobrinus*) or OMZ 918 (*S. mutans*) in mixed-species biofilms (table 5). However, higher counts of OMZ 918 were found in biofilms treated with sorbitol after feeding in comparison to biofilms exposed to xylitol after feeding (table 5).

Considering the delta CFUs values, with one exception no polyol effect on biofilms was observed. The exception concerns biofilms treated with sorbitol after feeding, whereby higher delta MS counts were found compared to the xylitol treatment before feeding (fig. 3).

Discussion

The present study was undertaken to test the hypothesis that the "futile xylitol cycle" could explain the postulated reduction of MS in dental plaque. The hypothesis stems from experiments with planktonic cells in monospecies cultures. This question was addressed using an oral polyspecies biofilm model simulating the meal pattern of man [van der Ploeg and Guggenheim, 2004; Thurnheer et al., 2006].

The mixed biofilms' response pattern to the four polyol treatments was similar for all eight MS strains tested (tables 2-5; fig. 3). With all treatments, differences in MS counts were minute, fluctuating mostly within one log-step. Expectedly, some variability in the ability of the MS strains to colonize the biofilms was found. The X-R strains colonized at a slightly lower level than the X-S strains, while the accompanying competing *S. oralis* strain was

favoured (tables 2, 3). The freshly isolated MS strains colonized the biofilms in higher numbers than the laboratory strains, at the expense of *S. oralis* (tables 4, 5).

The anticariogenic effect of xylitol has been claimed by many authors to be based on a reduction of MS in dental plaque [Söderling et al., 1989; Tanzer, 1995; Trahan, 1995; Mäkinen et al., 2005]. The present findings do not support that xylitol reduces MS counts in biofilms (tables 2-5, fig. 3). Further, conflicting data have been published and the clinical relevance of a reduction of the intra-oral load of MS is not clear [Beighton, 2005]. Not all studies concur with a mutans-reducing effect of xylitol [Birkhed et al., 1979; Giertsen et al., 1999; Van Loveren, 2004]. Short-term and long-term clinical studies with different designs, administration form, and dosage of xylitol show disparate findings [Van Loveren, 2004]. It should also be noted that there is no consensus as to an efficient or minimal "therapeutic" dose of xylitol [Van Loveren, 2004]. The concentration of xylitol used in the present study, and the repeated, limited time periods of exposures of biofilms to xylitol simulated the daily consumption or topical application of xylitol-containing products.

The energy-consuming futile cycle held responsible for the inhibitory effect of xylitol on MS involves uptake of xylitol via induction of a constitutive fructose-specific phosphotransferase transport system (PTS) [Assev and Rølla, 1984; Trahan et al., 1985; Assev and Rølla, 1986]. As a result, it was postulated that accumulated xylitol 5-phosphate will compete with phosphofructokinase and then arrest glycolysis by intracellular accumulation of glucose 6-phosphate [Assev and Rølla, 1986]. However, this cycle has only been proven to operate under highly un-physiologic conditions. In in vitro studies, pure cultures of *S. sobrinus* were first deprived of other carbohydrate reserves by incubation for 3 h with high concentrations of xylitol, whereupon the transport of only trace amounts of glucose was determined [Assev and Rølla, 1986]. Similarly, the formation of xylitol 5-phosphate in dental plaque was detected only after plaque samples had been incubated in vitro with xylitol

as the sole external carbohydrate source for 6 h [Wåler, 1992]. Thus, the bacteria had to grow under these conditions in order to induce the expression of the proteins necessary for this transport system, and the glucose concentration in the environment had to be very low in order to avoid catabolic repression [Carlsson, 1978]. Such conditions never prevail in the oral cavity and may also explain why xylitol did not inhibit MS under the present experimental conditions. Even after overnight starving, the concentrations of glucose in biofilms were obviously still sufficient to suppress the uptake of xylitol. Notably, to confirm the antimetabolic effect of xylitol in vivo intracellular accumulation of glucose 6-phosphate has not been demonstrated [Giertsen et al., 1999].

Thus, one may question whether oral microorganisms will, in fact, take up xylitol under in vivo conditions. The fructose-specific PTS has an anticipated Km for xylitol of 54 mM in contrast to 27 μM for fructose [Trahan et al., 1991], indicating that not until fructose levels are very low will xylitol be transported into X-S cells. The same holds for glucose. Glucose and fructose are the preferred hexoses of microorganisms. In saliva, glucose is always present at concentrations between 5 and 40 μM [Kelsey et al., 1972]. The concentration of glucose varies in plaque and due to enzymatic breakdown of extracellular glucans is most probably higher during "starvation" periods [Hotz et al., 1972]. Moreover, during food intake – as mimicked under the present experimental conditions – the glucose concentration in plaque is high and the pH low, favouring transport via alternative transport-systems [Carlsson and Hamilton, 1994].

We observed a slight, but statistically significant increase in MS numbers when biofilms were exposed after feeding to sorbitol (tables 2-5; fig. 3). This increase was accompanied by a slight, but statistically significant decrease in the numbers of *S. oralis*, and in some cases was also reflected in raised numbers of total CFUs and total streptococci (tables 2-5). It is well known that the majority of MS and lactobacilli, some *Actinomyces* sp., some

non-mutans streptococci, as well as other less frequently encountered oral microorganisms ferment sorbitol [Edwardsson et al., 1977; Havenaar et al., 1978; Kalfas and Edwardsson, 1990]. The reduction of *S. oralis* cells may thus be explained by the ability of MS and *A. naeslundii* to ferment sorbitol, resulting in an ecological advantage for *S. mutans* or *S. sobrinus* leading occasionally to higher total CFUs and streptococci counts in the biofilms, rather than by a sorbitol-induced inhibition of *S. oralis*.

The increased numbers of MS in biofilms exposed to sorbitol after feeding (tables 2-5, fig. 3) are in accordance with clinical studies reporting an increase in *S. mutans* counts in plaque following frequent consumption of sorbitol [Söderling et al., 1989] or frequent mouth rinses with a sorbitol solution [Kalfas et al., 1990]. Sorbitol is transported into bacterial cells using a phosphenol pyruvate (PEP)-dependent sorbitol PTS, resulting in intracellular accumulation of sorbitol 6-phosphate, which is then further converted to fructose 6-phosphate by sorbitol 6-phosphate dehydrogenase [Brown and Wittenberger, 1973; Slee and Tanzer, 1983]. Both enzymes are inducible, and in planktonic monocultures they are readily repressed by low levels of glucose, as normally found in the mouth [Slee and Tanzer, 1983]. Based on the common conception that the genotypic and phenotypic expression profiles of bacteria growing in biofilms are different from their planktonic counterparts, it may be postulated that gene regulation in biofilms induces the expression of enzyme activities necessary to transport and metabolize sorbitol only in the presence of glucose. This is supported by our observation that the sorbitol-induced increase in MS numbers was only discernible in biofilms when starved cells were exposed to sorbitol *after* feeding, indicating that the expression of this enzyme cascade in biofilms may in fact depend on a sufficient intracellular level of glucose. However, the slight increase in MS numbers occasionally observed in vivo and previously explained by adaptation to sorbitol is considered to be clinically insignificant [Birkhed and Bär, 1991; Bowen, 1996]. Fermentation of sorbitol is slow [Havenaar et al., 1978; Yamada et

al., 1985; Kalfas et al., 1990], resulting in pH drops in plaque above values that may cause dental caries [Birkhed and Edwardsson, 1978; Birkhed et al., 1979]. In contrast, glucose and sucrose in excess are rapidly catabolized mainly to lactate [Geddes, 1975; Carlsson and Hamilton, 1994]. Moreover, sorbitol does not promote plaque formation and adherence by synthesis of extracellular polysaccharides [Havenaar et al., 1978].

Claims of superior effects over other polyols from using xylitol in a variety of products are often supported by reference to laboratory findings of xylitol effects on planktonic bacteria. However, as shown in the present study, caution should be taken when trying to extrapolate from such un-natural conditions to possible events taking place in biofilms on teeth in vivo. Discrepancies between the present and previous in vitro findings may be explained by the intermittent exposures of multispecies biofilms to xylitol used in our study, mimicking more realistic conditions of consumption, in contrast to the exposure used in studies with planktonic monocultures where xylitol was included in the fluid medium throughout the experiments giving totally unrealistic exposure times [Assev et al., 1983; Assev and Rølla, 1984; Assev and Rølla, 1986].

In summary, the present study showed no evidence of xylitol-induced reduction of the cell numbers of X-S, X-R, freshly isolated, or laboratory MS strains grown in a multi-species biofilm environment. Analogously, total streptococci, or total CFUs remained unaffected in spite of repeated and prolonged exposures before or after biofilm feeding to relatively high concentrations of xylitol. These findings do not support the hypothesis that xylitol reduces the numbers of MS in dental plaque by futile metabolic cycles.

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Table headings

Table 1. Mutans streptococci strains used in mixed-six-species biofilms.

Table 2. Treatment effects on mixed-species biofilms containing a xylitol susceptible (X-S) mutans group strain.

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Table 4. Treatment effects on mixed-species biofilms containing a freshly isolated *S. mutans* strain.

Table 5. Treatment effects on mixed-species biofilms containing a laboratory reference strain of *S. sobrinus* or *S. mutans*.

Figure legends

Figure 1. Experimental arrangement and time flow of each biofilm experiment.

Figure 2. Schematic explanation of the allocation of biofilms to the different treatments in a single biofilm experiment. Each experiment was assigned one of the eight MS strains listed in Table 1.

Figure 3. Box-plot diagram showing the delta CFUs of biofilms according to polyol treatments.

Each box indicates the lower and the upper quartiles. Horizontal bars within boxes indicate median values. Data are based on eight distinct biofilm experiments.* Difference statistically significant by Scheffe test, $p < 0.05$.

Table 1. Mutans streptococci strains used in mixed-six-species biofilms

Strains	**Feature**
Streptococcus mutans CM7	xylitol sensitive (X-S)[1]
Streptococcus mutans CM7	xylitol resistant (X-R)[1]
Streptococcus mutans P-03	Fresh isolate
Streptococcus mutans M-51	Fresh isolate
Streptococcus mutans OMZ 918 (UAB159^T, ATCC 700610^T)	Laboratory strain[2]
Streptococcus sobrinus ATCC 27352	xylitol sensitive (X-S)[1]
Streptococcus sobrinus ATCC 27352	xylitol resistant (X-R)[1]
Streptococcus sobrinus OMZ 176	Laboratory strain[3]

[1] Strains kindly provided by Dr. Trahan, University of Quebec.
[2] Strain acquired from ATCC.
[3] Strain collection of the Section of Oral Microbiology, University of Zürich.

Table 2. Treatment effects on mixed-species biofilms containing a xylitol susceptible (X-S) mutans group strain

Treatments	*S. sobrinus* ATCC 27352 X-S strain				*S. mutans* CM7 X-S strain			
	Total CFUs	**Total streptococci CFUs**	***S. sobrinus***	***S. oralis***	**Total CFUs**	**Total streptococci CFUs**	***S. mutans***	***S. oralis***
Control	9.0 ± 2.8	5.3 ± 1.3	0.6 ± 0.08*	4.6 ± 1.4	5.9 ± 1.9*	3.8 ± 0.6*	3.3 ± 0.3*	0.7 ± 0.3
Xylitol before	7.6 ± 2.1	6.4 ± 1.9	0.7 ± 0.2	5.4 ± 1.9	5.2 ± 0.9*	4.4 ± 0.7*	3.2 ± 0.8*	1.2 ± 0.1
Xylitol after	7.8 ± 0.6	4.7 ± 1.5	0.6 ± 0.2	4.0 ± 1.2	9.8 ± 1.9	6.0 ± 3.5	3.3 ± 2.0*	2.6 ± 1.7
Sorbitol before	7.0 ± 0.3	5.2 ± 0.3	0.6 ± 0.05	4.7 ± 0.5	6.6 ± 2.3	6.6 ± 2.5	4.6 ± 1.9	1.7 ± 1.0*
Sorbitol after	9.7 ± 0.5	5.3 ± 1.4	1.0 ± 0.1	3.5 ± 0.7	14.0 ± 3.2	15.0 ± 1.5	15.0 ± 2.0	0.4 ± 0.2

Data are based on three separate biofilms and expressed as mean CFUs ± SD ($x10^8$). Significant differences (*) were observed only in comparison to the treatment "sorbitol after feeding", by Scheffe test ($p < 0.05$).

Table 3. Treatment effects on mixed-species biofilms containing a xylitol resistant (X-R) mutans group strain

Treatments	*S. sobrinus* ATCC 27352 X-R strain				*S. mutans* CM7 X-R strain			
	Total CFUs	**Total streptococci CFUs**	***S. sobrinus***	***S. oralis***	**Total CFUs**	**Total streptococci CFUs**	***S. mutans***	***S. oralis***
Control	15.0 ± 2.9	9.8 ± 3.9	0.2 ± 0.1	9.4 ± 4.1	11.0 ± 3.2	11.0 ± 0.5	0.5 ± 0.04*	9.8 ± 0.2*
Xylitol before	10.0 ± 5.0	8.3 ± 5.0	0.07 ± 0.01	8.3 ± 5.0	14.0 ± 1.5	12.0 ± 1.5	0.5 ± 0.03*	10.0 ± 1.8*
Xylitol after	9.7 ± 2.2	7.9 ± 3.8	0.1 ± 0.05	7.9 ± 3.8	8.9 ± 4.6	6.4 ± 1.9	0.7 ± 0.2*	5.6 ± 1.9*
Sorbitol before	6.6 ± 2.2	4.3 ± 0.7	0.07 ± 0.01	4.1 ± 0.8	10.0 ± 3.3	9.0 ± 1.2	0.9 ± 0.2*	7.7 ± 0.8*
Sorbitol after	7.3 ± 1.2	4.5 ± 0.7	0.07 ± 0.01	4.4 ± 0.7	9.0 ± 3.6	8.0 ± 4.3	7.8 ± 4.5	0.3 ± 0.3

Data are based on three separate biofilms and expressed as mean CFUs ± SD ($x10^8$). Significant differences (*) were observed only in comparison to the treatment "sorbitol after feeding", by Scheffe test ($p < 0.05$).

Table 4. Treatment effects on mixed-species biofilms containing a freshly isolated *S. mutans* strain

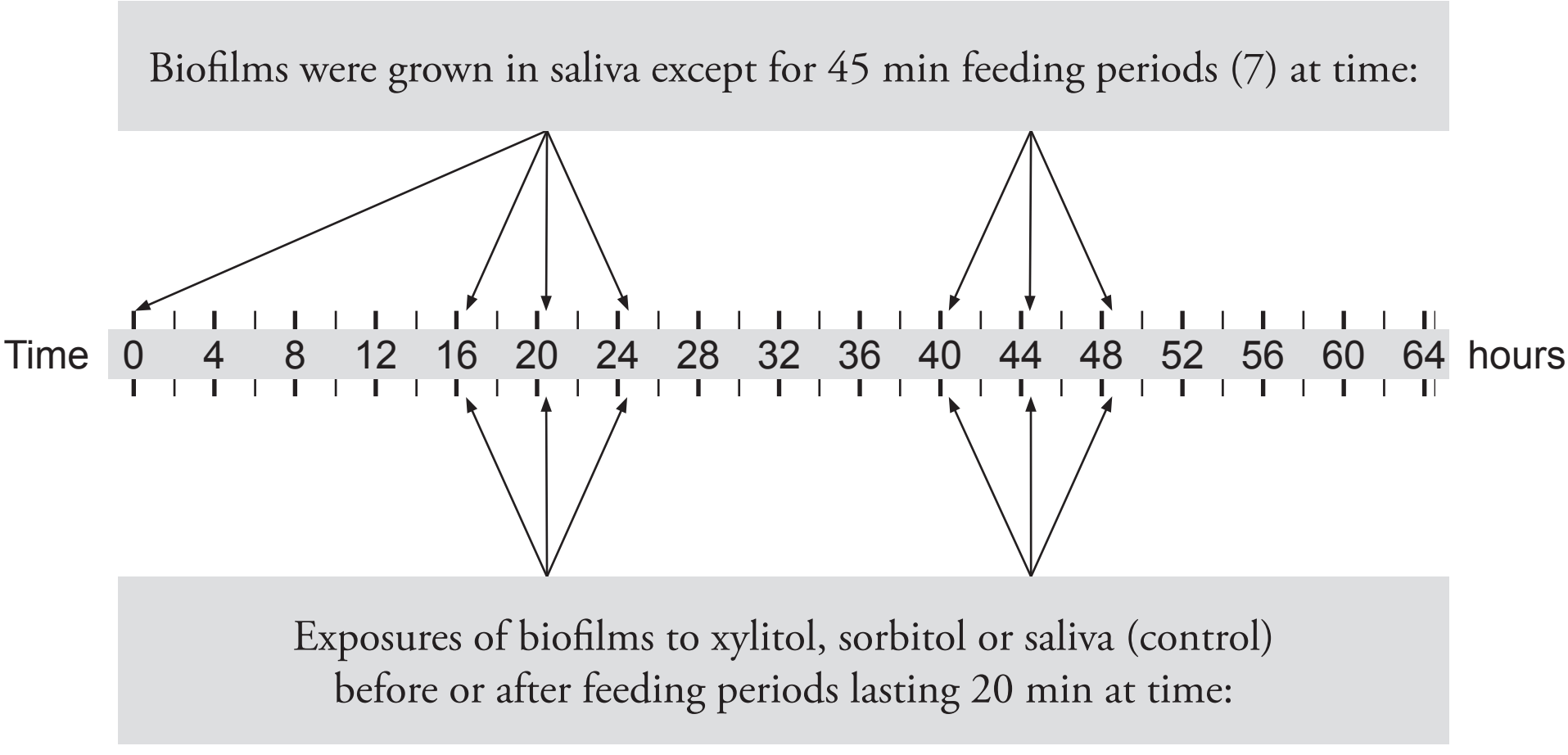
Treatments	Freshly isolated *mutans* P-03 strain				Freshly isolated *mutans* M-51 strain			
	Total CFUs	**Total streptococci CFUs**	***S. mutans***	***S. oralis***	**Total CFUs**	**Total streptococci CFUs**	***S. mutans***	***S. oralis***
Control	7.7 ± 1.4	7.2 ± 1.7	4.0 ± 0.5	3.2 ± 1.2	4.7 ± 2.5	4.5 ± 3.6	3.7 ± 3.2	0.4 ± 0.1*
Xylitol before	5.6 ± 2.4	2.7 ± 2.1	1.4 ± 1.4	1.4 ± 0.7	2.8 ± 1.4	2.1 ± 0.7	1.9 ± 0.6	0.2 ± 0.02*
Xylitol after	6.9 ± 1.9	5.2 ± 1.7	3.4 ± 0.6	1.9 ± 1.0	6.5 ± 1.6	7.3 ± 2.1	7.0 ± 2.0	0.4 ± 0.02*
Sorbitol before	6.3 ± 1.9	4.5 ± 1.1	2.7 ± 0.7	1.8 ± 0.4	6.3 ± 3.2	5.2 ± 1.6	4.9 ± 1.6	0.5 ± 0.1*
Sorbitol after	8.8 ± 1.9	7.3 ± 0.8	5.3 ± 0.7	2.1 ± 0.7	8.5 ± 1.2	6.4 ± 0.8	6.4 ± 0.8	0.02 ± 0.01

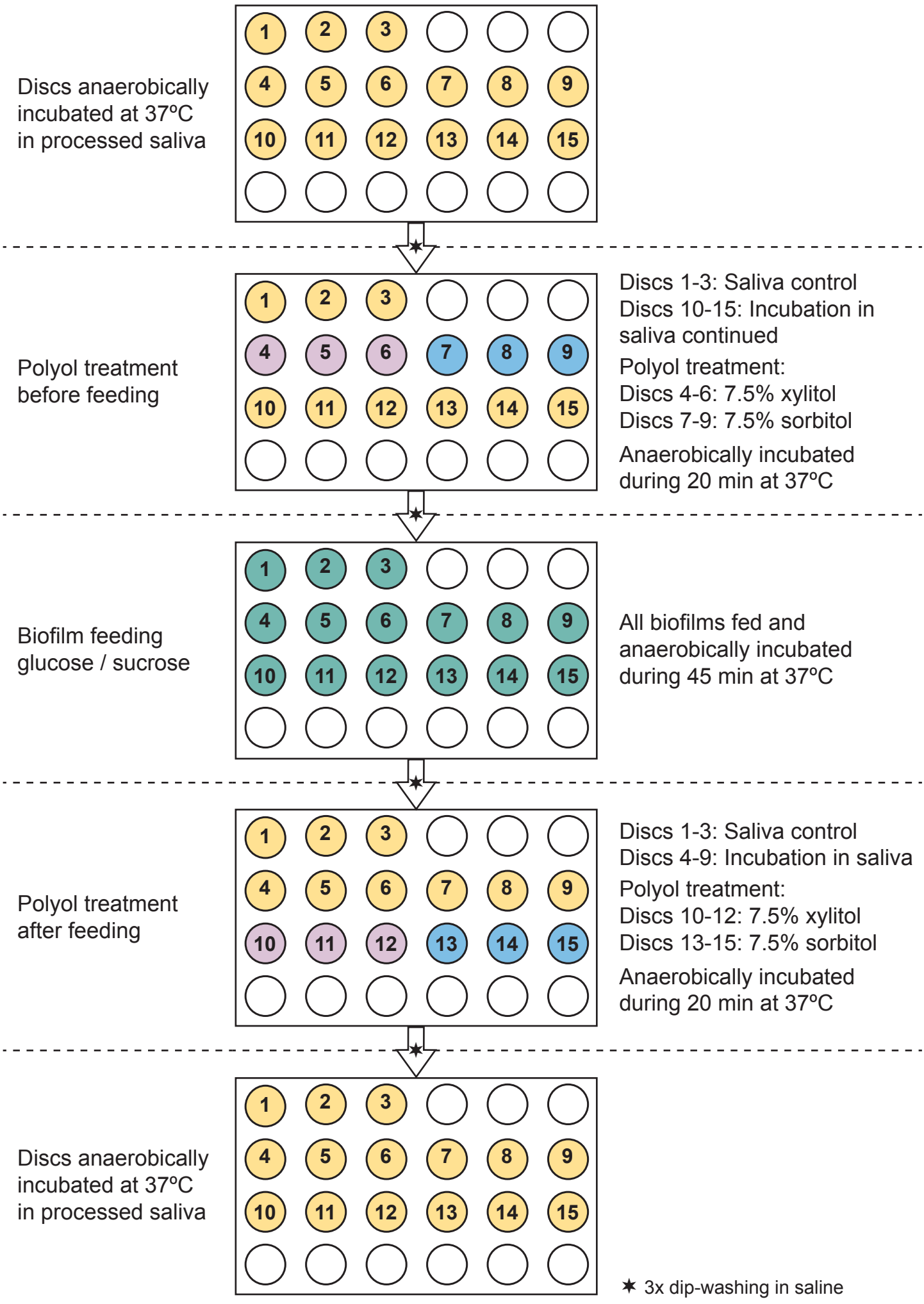
Data are based on three separate biofilms and expressed as mean CFUs ± SD ($x10^8$). Significant differences (*) were observed only in comparison to the treatment "sorbitol after feeding", by Scheffe test ($p < 0.05$).

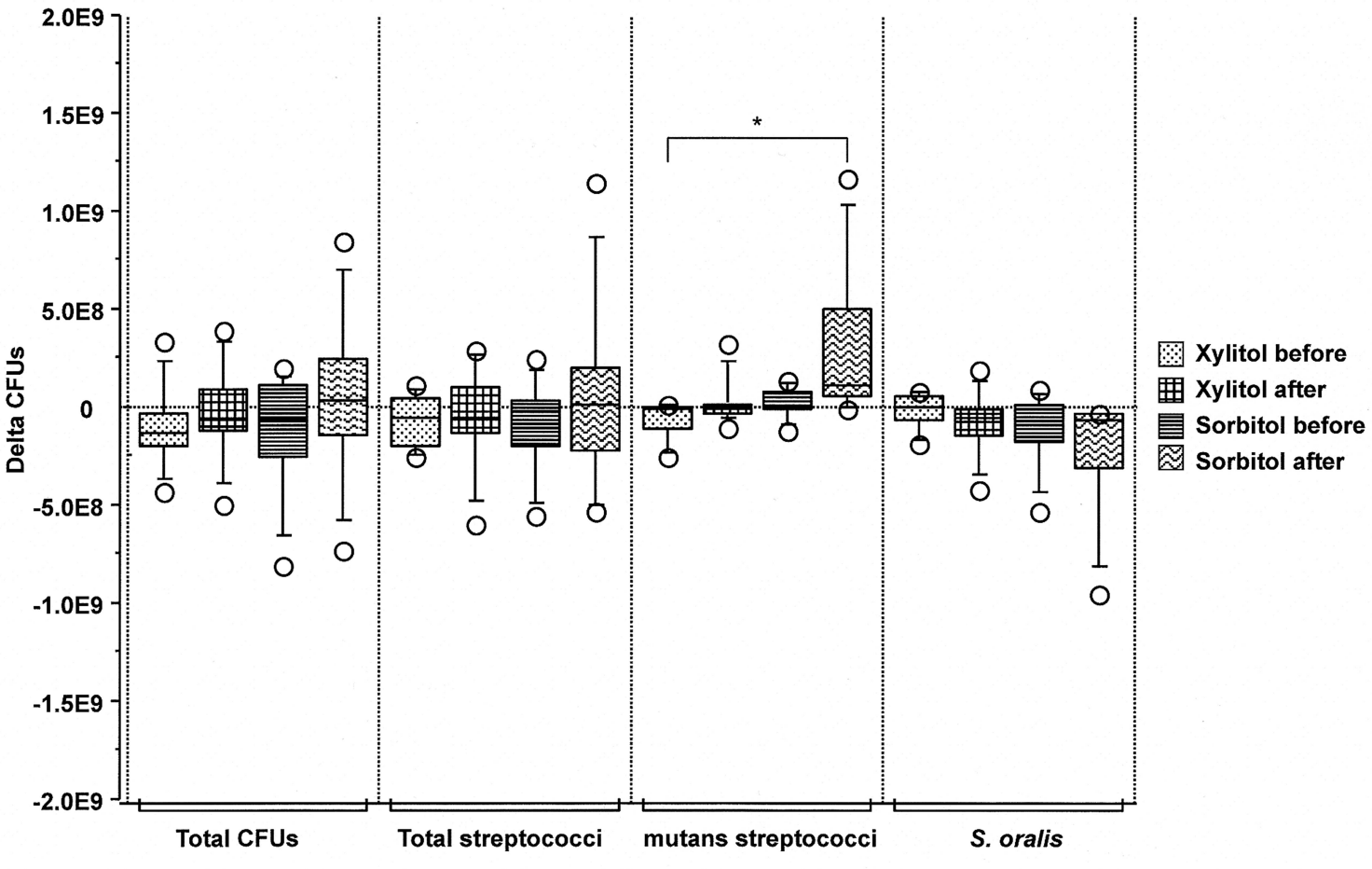
Table 5. Treatment effects on mixed-species biofilms containing a laboratory reference strain of *S. sobrinus* or *S. mutans*.

Treatments	Laboratory *sobrinus* OMZ 176 strain				Laboratory *mutans* OMZ 918 strain			
	Total CFUs	**Total streptococci CFUs**	***S. sobrinus***	***S. oralis***	**Total CFUs**	**Total streptococci CFUs**	***S. mutans***	***S. oralis***
Control	2.7 ± 0.4	1.1 ± 0.1	0.5 ± 0.3	0.6 ± 0.3	6.2 ± 1.2	1.9 ± 0.2	1.2 ± 0.3	0.7 ± 0.1
Xylitol before	2.6 ± 0.1	1.4 ± 0.4	0.4 ± 0.3	0.9 ± 0.1	4.9 ± 1.8	1.4 ± 0.3	0.8 ± 0.1	0.5 ± 0.3
Xylitol after	1.9 ± 0.6	0.7 ± 0.2	0.5 ± 0.2	0.2 ± 0.04	4.9 ± 1.6	1.2 ± 0.2	0.7 ± 0.2*	0.5 ± 0.1
Sorbitol before	2.6 ± 0.8	1.1 ± 0.3	0.4 ± 0.05	0.7 ± 0.2	3.1 ± 1.6	1.7 ± 1.4	1.2 ± 0.7	0.4 ± 0.5
Sorbitol after	1.9 ± 0.8	1.2 ± 0.2	0.8 ± 0.1	0.3 ± 0.1	4.0 ± 0.8	2.1 ± 0.2	2.0 ± 0.2	0.4 ± 0.1

Data are based on three separate biofilms and expressed as mean CFUs ± SD ($x10^8$). Significant differences (*) were observed only in comparison to the treatment "sorbitol after feeding", by Scheffe test ($p < 0.05$).


Biofilms were grown in saliva except for 45 min feeding periods (7) at time:
Time
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64
hours
Exposures of biofilms to xylitol, sorbitol or saliva (control)
before or after feeding periods lasting 20 min at time:


Discs anaerobically incubated at 37ºC in processed saliva
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Polyol treatment before feeding
Discs 1-3: Saliva control
Discs 10-15: Incubation in saliva continued
Polyol treatment:
Discs 4-6: 7.5% xylitol
Discs 7-9: 7.5% sorbitol
Anaerobically incubated during 20 min at 37ºC
Biofilm feeding glucose / sucrose
All biofilms fed and anaerobically incubated during 45 min at 37ºC
Polyol treatment after feeding
Discs 1-3: Saliva control
Discs 4-9: Incubation in saliva
Polyol treatment:
Discs 10-12: 7.5% xylitol
Discs 13-15: 7.5% sorbitol
Anaerobically incubated during 20 min at 37ºC
Discs anaerobically incubated at 37ºC in processed saliva
✶ 3x dip-washing in saline


2.0E9
1.5E9
1.0E9
5.0E8
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-5.0E8
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-2.0E9
Delta CFUs
*
Total CFUs
Total streptococci
mutans streptococci
S. oralis
Xylitol before
Xylitol after
Sorbitol before
Sorbitol after